Scientific Note

Effect of the Guppy, *Poecilia reticulata*, on Oviposition of *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract. Many mosquito species avoid oviposition sites infested with predators of their progeny. We investigated whether the guppy *Poecilia reticulata* Peters affected the oviposition site selection of natural blood-fed gravid *Culex qinquefasciatus* Say females in both the laboratory and field. In the laboratory, there was no significant difference between the average numbers of egg rafts laid by *Cx. qinquefasciatus* females on water with fish and without fish. In the field, there was no significant difference between the average numbers per night of gravid *Cx. qinquefasciatus* females collected from gravid traps with fish and without fish. Our investigation shows that natural blood-fed gravid *Cx. quinquefasciatus* does not avoid oviposition sites with *P. reticulata*. The phenomenon of absence or very low number of mosquito larvae in some water habitats in the presence of *P. reticulata* is discussed.

Key words: *Poecilia reticulata*, *Culex quinquefasciatus*, oviposition, predator

Introduction

The guppy, *Poecilia reticulata* Peters, was introduced into Hawaii in 1922 for mosquito control (Brock 1960). Some studies show that many mosquito species avoid oviposition sites infested with predators of their progeny as they can detect the chemical cues produced by predators (Petranka and Fakhoury 1991, Ritchie and Laidlaw-Bell 1994, Angelon and Petranka 2002, Munga et al. 2006, Van Dam and Walton 2008). The Southern House Mosquito, *Culex quinquefasciatus* Say, is one of the six invasive pest mosquitoes in Hawaii, mainly breeding in ground water such as ditches, ponds, etc. (Hawaii Department of Health, Vector Control Branch 1991). There are conflicting reports about responses of gravid *Cx. quinquefasciatus* females to chemical stimuli of its predators. Louca et al. (2009) reported that ovipositing *Cx. quinquefasciatus* females avoided water with *Tilapia quineensis*, a native fish species in the Gambia. Angelon and Petranka (2002) indicated that they could detect chemicals from the Western mosquito fish, *Gambusia affinis*, if the amount of the chemicals was above a threshold. Van Dam and Walton (2008) reported that their oviposition was slightly reduced in water with fish exudates, but was not consistently deterred by water conditioned by *G. affinis*. Other reports showed that they did not respond to the presence of chemicals exuded from *G. affinis* (Walton, et al. 2009) or from *Melanotaenia duboulayi*, a larvivorous fish species (Hurst et al. 2010) in oviposition sites.

In the past when we took samples from different types of potential breeding sites of *Cx. quinquefasciatus*, such as ditches, ponds on Oahu, we always found absence or very low number of mosquito larvae if *P. reticulata* was present. No studies have been performed to examine the effect of *P. reticulata* on oviposition of *Cx. quinquefasciatus*. It would be helpful to understand the effect of *P. reticulata* on *Cx. quinquefasciatus* female oviposition when developing mosquito control strategies. Here we investigated in the laboratory and in the field whether natural blood-fed gravid *Cx. quinquefasciatus* females would avoid ovipositing in water where *P. reticulata* was present.

Materials and Methods

Source of mosquitoes. Gravid traps (Hausherr's Machine Works, Toms River, NJ) baited with 10 liters of infused water were set up at Honolulu International Airport area, and in the yard of the Vector Control facility, Oahu, Hawaii. The traps were set up between 0800 to 0930 h and gravid *Cx. qinquefasciatus* females were collected between 0800 to 0830 h the next morning. The infused water was made by adding approximately 100 g hay and 60 g dry horse manure to 20-liter (5-gallons) of tap water, and allowing the mixture to ferment for 4 days. All naturally blood-fed gravid female mosquitoes were collected in November and December, 2013.

Source of guppies. The guppies, *P. reticulata* were obtained from colonies maintained at the Vector Control Section's facility, Halawa Valley, Aiea, Oahu.

Laboratory experiment. Twenty five to thirty wild-caught gravid female mosquitoes (abdomen becoming dilatation and whitish) were randomly chosen and transferred with an aspirator into a cubical screen cage (30 cm x 30 cm x 30 cm). For each treatment, one adult female *P. reticulata* was placed into a cylindrical plastic container (10 cm high, 11 cm in diameter) filled with 8 cm of tap water for three days before being exposed to mosquitoes. Preliminary experiments showed that *P. reticulata* ate an average 1.7 ± 1.6 (±SE) *Cx. quinquefasciatus* egg rafts within 24 hours. Based on this preliminary experimental result, an 11-cm diameter metal screen (1/8 inch pore-size) was set up as a barrier midway up the cylinder such that *P. reticulata* was under the screen and unable to eat the mosquito egg rafts on the water surface. The average size (±SD) of *P. reticulata* in the study was 3.1 ± 0.1 cm. As a control, we used a similar container with tap water and a metal screen in the middle but no fish. Each trial was started when both treatment and control containers were placed and exposed to the source of female mosquitoes inside the cubical screen cage prepared as mentioned above. Egg rafts laid by the mosquito females were counted and removed daily for 7 days. The total number of egg rafts was calculated in the end of the test. The experiment was repeated 8 times in November and December 2013, using a fresh batch of gravid mosquitoes and new fish each time. During the experiment, adult mosquitoes were fed with a 5% sucrose solution presented in a cotton wick in a vial, and the cages were held in a room with metal screen wall, receiving both artificial and natural light and at ambient temperature.

Field experiment. We investigated whether *P. reticulata* affected the number of gravid *Cx. qinquefasciatus* females being attracted to gravid traps in the field. Two gravid traps were baited with a 4-day-old infusion of hay and tap water (100 g hay in 9.5 liters of water) and were set up 2 m apart in the yard of the Vector Control facility, Aiea, Oahu. Ten *P. reticulata* chosen randomly from the guppy colony at the Vector Control facility were introduced into one gravid trap directly so that this gravid trap with guppies became the treatment. No guppy was introduced to the other one so that it became the control.

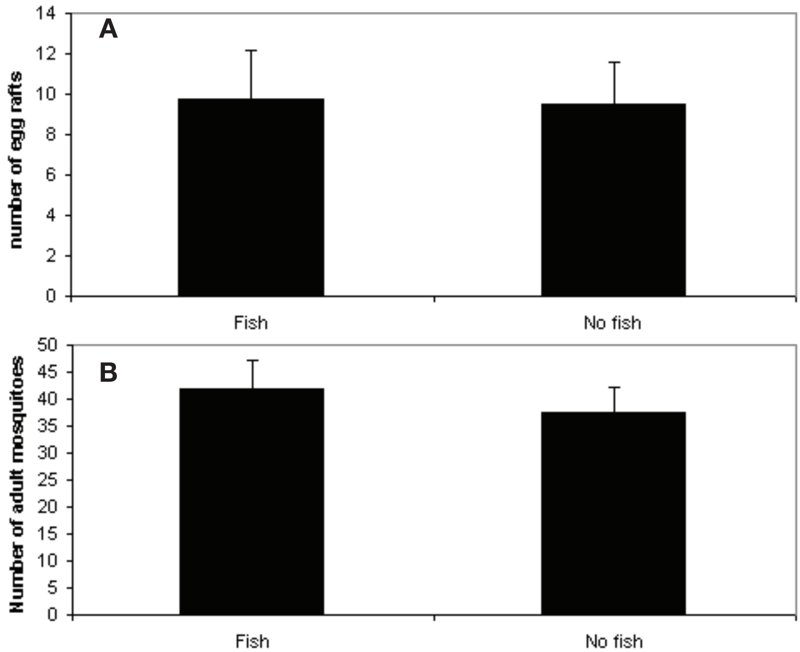

Figure 1. (A) Average numbers (±SE) of egg rafts laid by *Culex quinquefasciatus* in treatment (with one mosquito fish) and control (without mosquito fish), and (B) average per night (±SE) of gravid *Culex quinquefasciatus* females collected in gravid traps (treatment: 10 mosquito fish; control: without mosquito fish). Both vertical bars represent standard error.

The traps were operated daily from 4:00 pm to 8:00 am from Monday to Friday. Collection nets were changed each morning and gravid *Cx. qinquefasciatus* females were collected and counted in the process. The infused water and mosquito fish were changed weekly and at the same time the positions of treatment and control were rotated. The test was carried out for 17 nights in November and December, 2013.

Data analysis. Both average numbers of egg rafts laid by the gravid *Cx. qinquefasciatus* females in the Laboratory, and average numbers per night of gravid *Cx. qinquefasciatus* females obtained in the field were calculated and the comparisons were examined using t-tests. All data were transformed by square root before the analyses.

Result and Discussion

For the laboratory oviposition site selection test, the average numbers of egg rafts laid by the mosquitoes in the treatment and control sites were not significantly different (Fig. 1A; t-test: t=0.945; df=14; p>0.05). For the field test, the average numbers per night of gravid mosquito females attracted to the gravid traps in the treatment and control were also not significantly different (Fig. 1B; t-test: t=0.614; df=32; p>0.05). Both tests indicate that *Cx. quinquefasciatus* did not avoid ovipositing in sites containing *P. reticulata*. This result generally agrees with other reports that *Cx. quinquefasciatus* does not avoid oviposition sites with predators, or their chemical cues (Hurst et al. 2010, Walton et al. 2009). *Culex quinquefasciatus* breeds in different types of ground water, but most heavily in water with high organic content, such as polluted ground pools, open septic tanks, and effluent drains from sewage disposal plants (U.S. Department of Health and Human Services 1987). In our routine mosquito control practice, we never found the fish *P. reticulata* in this kind of high-organic-content habitats in Hawaii, even though it is more tolerant of pollution than *G. affinis*. Also, the fish was not present in natural or artificial habitats such as tree holes, buckets etc., where *Culex quinquefasciatus* usually breeds. Therefore, larval habitats of *Cx. quinquefasciatus* are generally sites with low risk of fish predation, and Hurst et al. (2010) suggested that fish would not be a strong selective agent for this species from an evolutionary standpoint.

The absence or low numbers of mosquito larvae that we have observed in water with *P. reticulata* is believed mainly due to predation by the guppies, as supported by the data of these experiments. Bay and Self (1972) also reported that *P. reticulata* can discover and destroy *Culex pipiens fatigans* (= *quinquefasciatus*) larvae even at low densities in some habitats in Rangoon, Burma. Obviously, *P. reticulata* have played an important role in mosquito control in Hawaii, as *Cx. quinquefasciatus* do not avoid oviposition in habitats with natural guppy populations.

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